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THE INHERITANCE OF RESISTANCE OF WHEAT SPINDLE STREAK MOSAIC VIRUS (WSSMV) IN WINTER WHEAT

By

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ABSTRACT

THE INHERITANCE OF RESISTANCE TO WHEAT SPINDLE STREAK MOSAIC VIRUS (WSSMV) IN WINTER WHEAT

By

Mark Van Koevering

The inheritance of resistance to wheat spindle streak mosaic virus (WSSMV) was studied in a seven parent diallel analysis. Parents were chosen for the diallel based on their differential reaction to the disease.

The plants were inoculated with virus by using soil infested with the fungal vector, *Polymyxa graminis*. Immunologically specific electron microscopy (ISEM) was developed by Haufler and Fulbright (10) to sample the wheat plants and rate them based on the actual number of virus particles.

Resistance to wheat spindle streak mosaic virus was found to be a qualitative trait controlled by only two genes. The genetic variation for resistance to WSSMV in the F_1 progenies and parental populations appears to be primarily due to dominant gene action. Additive and epistatic gene action was also apparent among susceptible populations. In addition, the fungal vector was found in all genotypes, including resistant germplasm. Therefore, resistance to WSSMV is not due to the plant's resistance to the fungal vector.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

INTRODUCTION

Virus diseases have recently caused considerable damage to soft winter wheat (Triticum aestivum em Thell L.) in the Eastern United States. One of the most common diseases has been wheat spindle streak mosaic virus (WSSMV). WSSMV is vectored by a common soil-borne fungus, Polymyxa graminis. The vector transmits the virus to susceptible plants by infecting wheat roots in the fall. WSSM symptoms are apparent in the early spring when temperatures remain cool and first appear in lower leaves as long yellow streaks or dashes which taper at both ends. The disease reduces grain yield in winter wheat by limiting the number of seed producing tillers in infected plants. Although WSSMV was identified by Slykhuis (25) in 1960, only recently has the incidence of the disease increased to the point of economic significance. Its rise to importance has presented several problems.

Increasing infestation has caused great concern among farmers and scientists, particularly since most cultivars grown throughout the Eastern United States are susceptible to the virus. In addition, there is very little information available about resistant germplasm, and more specifically, the mode of inheritance of resistance to WSSMV and the gene action among resistant alleles. Therefore, it was necessary to begin a detailed genetic study concerning WSSMV.

The study had three main objectives:

1) To evaluate the germplasm for resistance to WSSMV;
2) To determine the mode of inheritance and gene action of resistance;
3) To prepare a breeding strategy to introduce resistance into an acceptable variety.

The germplasm was screened for WSSMV resistant lines by evaluating foliar symptoms in the field and using serological techniques in the laboratory. Parents were chosen for the genetic study based on their differential reactions to the virus. The genetic study used a diallel analysis to investigate the parental germplasm. An additional study focused on the fungal vector, and its relationship to the resistant lines.

Together, these two studies provided an insight into the inheritance of resistance, the gene action of resistant alleles, and the role of the fungal vector. These results should improve the wheat breeding program by identifying useful WSSMV resistace and providing specific knowlege of the genetic systems involved in resistance to the virus.

LITERATURE REVIEW

Wheat spindle streak mosaic (WSSM) is an unique mosaic disorder that affects winter wheat. Since 1960, when WSSM was first identified (25), little progress has been made in understanding the disease, the vector, and the environmental interactions between host, vector, and virus. This literature review will present the results and significance of past research efforts and explore the many questions that remain unanswered.

Geographical Distribution

Since the first report in Canada (25), WSSM has been observed throughout the soft winter wheat growing regions of the Eastern United States. Researchers in Michigan (38) reported a wheat variegation disorder in 1970 that was later identified as WSSM. In the United States, outbreaks of WSSM have been reported and confirmed in the states of Indiana (16), Kentucky (40), Pennsylvania (22), Maryland, New York (31), and Nebraska (4). Internationally, occurrence of the disease has been documented in Southern France (31) and in India (1). Most reports described widespread and uniform infections. This suggests that WSSM was present years before being accurately identified. One might ask why WSSM is suddenly appearing throughout many states and countries. There are several plausible explanations.

First, WSSM symptoms may have been masked by other diseases producing similar symptoms (26). For example, wheat streak mosaic (WSM) and Agropyron mosaic (AM) are easily confused with WSSM. In addition, WSSM symptoms on severely infected plants may appear very similar to the intensely chlorotic symptoms common to Septoria leaf blotch. Besides the possibility of confusing other diseases with WSSM, foliar symptoms of WSSM have often been mistakenly attributed to cold temperature damage (16). The difficulty in isolating this particular plant virus for accurate identification has magnified the problem of reporting disease outbreaks.

In addition to the frequent misidentification of WSSM, the use of new susceptible cultivars greatly increased the potential for infection (17). Almost all of the new cultivars are susceptible to WSSMV, while older cultivars may show some resistance (16, 22, 26, 39, 40). In light of the increased infestation, researchers have begun to explore the disease in more detail.

Host Range and Symptomology

Initially, researchers sought to accurately describe the symptoms and determine the host range of WSSM. Symptoms appear in the spring, when growth is initiated. Light green to yellow streaks, or spindle-shaped dashes, parallel to the leaf axis appear throughout the leaf blade and develop from the leaf tip to its base. The symptoms are more evident on the lower leaves, but will continue to develop on upper leaves under favorable conditions. If the weather remains ideal for disease development, the chlorotic lesions increase, diffuse, and coalesce into large non-distinct mosaic patterns. Continued favorable environmental conditions allow the disease to progress to the flag leaves of susceptible lines and symptoms may remain even after heading (8, 16, 25, 26, 38, 39, 40). An accurate description of the foliar symptoms facilitated the determination of possible hosts.

WSSM only affects winter wheat and durum wheat (*Triticum durum* L.). Most of the commonly grown wheat cultivars are susceptible to WSSM (16, 17, 22, 32, 37, 39). Other species of the family *Gramineae* do not develop symptoms (17, 26, 32). Some of the species tested include *Agropyron repens* (L.) Beauv, *Avena sativa* L., *Bromus inermis* Leyss., *Dactylis glomerata* L., *Hordeum vulgare* L., *Lolium multiflorum* L., *Phleum pratense* L., *Poa pratensis* L., *Secale cereale* L. and *Zea mays* L. In addition, *Amaranthus retroflexus* L., *Chenopodium album* L., *Trifolium pratense* L. and a number of other dicotyledonous plants did not exhibit local lesion symptoms (32).

Transmission of WSSMV

Most plant viruses are transmitted by a vector. A common soil-borne fungus, Polymyxa graminis (3, 23, 33), is reported to be the vector of WSSMV. Several zoosporic fungi are endemic in the wheat growing regions of the United States, and several of these fungi occur in fields infested with WSSMV. The most commonly found fungi include Polymyxa graminis, Olpidium brassicae, Rhizophydium graminis, Lagena radicicola and Pythium species (3, 23). All of these fungi could be considered suspect, since they are capable of surviving in dried soils and infecting wheat roots during periods of high osmotic potential.

In order to determine which fungi transmit the virus, Slykhuis and Barr (33) maintained purified fungal strains isolated from the roots of infected wheat plants. Seedlings of susceptible wheat lines were germinated in sterilized soil and infected with WSSMV by sap inoculation of the leaves. The roots of the WSSMV infected seedlings were then inoculated individually with the different fungal strains. The fungal strains were recovered from the WSSMV infected wheat and transferred to healthy wheat seedlings. Only plants infected with P. graminis transmitted the virus by root association to uninfected wheat plants. Other studies (3, 23) correlated the presence of P. graminis with the appearance of WSSMV but not with the concentration of P. graminis in the roots of infected plants. Therefore, infection was independent of the inoculum dosage, which suggested that not all zoospores were virulent or equally competent to transmit WSSMV (23).

A variety of environmental factors influence transmission of WSSMV by P. graminis, but the most important effect is temperature (26, 27, 33). The temperature requirements for vector and virus replication appear contradictory. For example, P. graminis develops most rapidly at temperatures between 15 and 22 C, but slowly at 10 C. On the other hand, WSSM symptoms develop most rapidly at a temperature of 10 C, and symptoms disappear at temperatures greater than 15 C (16, 17, 23, 26, 27, 30). However, rather than being contradictory, this data suggests that infection occurs in the fall soon after wheat emerges and requires temperatures in the range of 10 to 22 C to assure the build-up of a fungal population and root infection. Later in the season, temperatures decrease, facilitating virus replication and symptom development (28). Wiese and Hooper (37) noted a "vernalization" effect which stimulated root infection. Slykhuis (31) provided further evidence for this in 1976. Together, these studies concluded that the transmission of WSSMV to wheat seedlings was promoted by cool and freezing temperatures. Low temperatures increased both symptom severity and the incidence of infection by damaging the roots of young seedlings. Wiese and Hooper's study also showed that cold treatments outdoors were more effective than growth chamber cold treatments at 1 C. This was probably due to the freezing-thawing action of the soil which damaged the roots of susceptible hosts, increased vector infection and transmission of WSSMV.

Soil moisture is another important environmental factor influencing the transmission of WSSMV. Wet soil taken directly from a WSSMV infested field does not facilitate effective transmission of the virus to host plants (30, 37). Since keeping soil moist has a temporarily suppressive effect on infectivity, air-dried soil is a much more efficient media for virus transmission (37). However, soil moisture is important for the spread of fungal zoospores in the soil, since the zoospores are transported via water (31). Although zoospores of the fungus are found on very small clay particles in the soil (26), soil texture does not play a specific role in virus transmission, because all soil types transmit WSSMV to susceptible wheat seedlings (3, 26). However, in cold frame experiments in which heavier soils were used, enhanced virus infection occurred when sterilized sand was added to the infested soil which presumably increased drainage and aeration (27).

Seasonal Transmission

Virus transmission and replication is seasonal due to the importance of soil temperature and moisture. Infection occurs in the fall soon after emergence and continues throughout the winter in susceptible lines (26). Freezing and thawing increase root susceptibility to infection from the soil-borne fungus. In the spring, virus replication occurs when temperatures remain between 8-12 C.

Since it is known that early planting increases the amount of virus in susceptible cultivars (16, 22), late planting was suggested as a possible cultural means of controlling the disease. Late planting reduces disease incidence, but yield losses are usually greater for late planted wheat than for early planted material (22, 26, 28) due to an increase in winter kill when wheat is planted late.

Crop rotation is another cultural practice which might effectively reduce WSSMV infection. Fields in which wheat was planted continuously showed an increased incidence of infection (16, 26). This was most likely due to an increase in the vector inoculum in the soil. In fields where wheat was not grown for several years, infection still occurred when wheat was replanted (3, 26). Therefore, crop rotation is not an effective means of preventing the occurrence of WSSMV.

Soil and Mechanical Transmission

In order to study the disease further, a uniform infection procedure was required. Several attempts at mechanical transmission have been successful (17, 29, 26), but the procedure is difficult and unreliable. Sporadic infection was achieved and virus concentration within the plant tissue remained very low. Therefore, soil transmission has remained the preferred method of obtaining virus infection because of the efficient and uniform infection results. In addition, higher rates of infection occurred when the plants were vernalized in an outdoor coldframe (31, 37). After vernalization, the plants were grown at 10 C to enhance virus replication as represented by optimal symptom production.

Effects

WSSM is prevalent throughout the Midwest's soft winter wheat growing regions. It is the most obvious wheat disease in spring and early summer. Wiese *et al.* (39) estimated through growth chamber studies that yields were reduced 3 to 18% each year in Michigan. All wheat yield components were negatively affected by WSSM in their studies. The most notable change in yield components was a reduced number of tillers. Both the number of tillers and the tillers producing seed were significantly reduced (22, 26, 39). Other traits that were shown to be affected included plant height, straw yield, and plant vigor (22, 26). In 1970, a statewide infection of 10-30% and yield losses estimated to be 2-6% were reported in Michigan (39).

WSSM Identification

Until 1983, WSSM was diagnosed by foliar symptomatology, leaf-dip preparations, and the presence of pinwheel inclusion bodies in leaf bundle sheath cells (37). Infected tissue has a low virus concentration which made leaf-dip preparations inaccurate, and the preparation of leaf ultrathin sections was expensive and time consuming. In addition, rating breeding lines based on symptoms alone can be misleading, since they are often confused with other diseases (17, 26). Although field rating systems may be helpful for a broad survey of germplasm, the data are subjective and must be treated as such. Therefore, a sensitive and specific assay was needed to quantify the amount of WSSMV.

Advanced diagnostic techniques to detect viruses using electron microscopy were described by Derrick in 1973 (5). Later, Haufler and Fulbright (10, 11) perfected the use of immunological specific electron microscopy (ISEM) to detect WSSMV infection in plants. This technique has been a valuable tool in diagnosing and researching WSSMV. ISEM offers an accurate means of quantifying virus concentrations in different cultivars.

Purification and Serology

Prior to using ISEM, the long, flexuous virus rods were difficult to find in leaf ultrathin sections and leaf-dip preparations (17, 37). Attempts to purify WSSMV had been unsuccessful until 1979 when Usugi and Saito (36) first reported the purification of WSSMV. In 1983, Haufler and Fulbright (10) purified WSSMV by grinding infected wheat tissue in liquid nitrogen, extracting in buffer with various amendments, and concentrating in polyethylene glycol, followed by two cycles of ultracentrifugation through a sucrose-cesium sulfate density gradient. The purified virus was injected into rabbits to obtain an antiserum specific to WSSMV. Once the antiserum was isolated, ISEM procedures were developed. The antiserum was used to coat plastic and carbon-coated copper grids. The treated grids were placed on drops of infected tissue extract, then treated again with antiserum, negatively stained, and viewed with a transmission electron microscope (10). Haufler _et al_. (12) have used this technique to evaluate germplasm from both field and growth chamber plants.

Resistance to WSSMV

Various degrees of symptom severity have been found among many wheat lines screened for resistance to WSSM in the field (16, 17, 26, 38, 40). Haufler _et al._ (12) examined five commercial cultivars (Augusta, Ionia, Genesee, Pioneer S-76, and Tecumseh) and ten experimental lines under field and growth chamber conditions. Unlike other WSSM programs, these ratings were based on both symptom severity and virus particle counts using ISEM. Several lines resistant to the virus were identified. However, the genetic mechanisms controlling the expression of resistance still were unknown.

It is unclear whether the host prevents fungal infection of the roots, or inhibits virus replication within the plant. Many plants which are attacked by viruses transmitted by zoosporic fungi resist infection by blocking the vector. Such hosts are considered resistant to the disease but are not necessarily resistant to the virus, since they can still be infected by mechanical inoculation.

In 1984, Larson et al. (20) studied two plant viruses, wheat streak mosaic virus (WSMV) and soil-borne wheat mosaic virus (SBWMV), which are transmitted by mites and zoosporic fungi respectively. They found fungal vectors in all lines tested, including resistant material. In another study, the WSSMV "resistant" cultivar "Monon" developed symptoms after mechanical inoculation (17). However, Monon was not examined for the presence of the fungal vector. These results were not based on serological studies, but rather on symptomatology and electron microscopy of ultrathin sections of leaf material. This is significant, since even when plant symptoms are not visible, plant growth and yield can be significantly reduced from WSSMV infection (22). In addition, serological techniques are much more sensitive than traditional non-serological methods (5) and if used may have detected the presence of WSSMV. Although this does not refute earlier evidence suggesting that resistance is obtained by blocking the vector, some doubt still remains.

A genetic study of resistance to WSSMV is lacking. Several resistant sources of germplasm are available (12, 16, 17, 22, 26, 39, 40), although the data are based on various criteria. That is, some lines were determined resistant simply by the lack of foliar symptoms (22, 39, 40), others by the absence of pinwheel inclusion bodies (16, 17, 26), and most recently, resistance has been determined by counting the number of virus particles using ISEM (12). Clearly, ISEM offers the most sensitive and accurate means of rating germplasm for a genetic study.

Genetic Study of Resistance

The nature of inheritance of resistance to WSSMV is not known. A diallel analysis can provide statistics to investigate this genetic system. The diallel has been used in numerous studies and has been the subject of much controversy (2, 9, 13, 18, 21, 35). It is not the purpose of this paper to review all of the literature concerning diallels. Rather, the assumptions for the successful interpretation of a diallel will be discussed.

A diallel cross is the set of all possible matings among several parents (13). The diallel provides statistical measurements of additive and dominance variation, relative variation of the parental lines, and direct non-allelic genic interaction (13, 18). One item of contention is the model itself. The issue concerns whether the parental population should be considered as a random sample from some larger population (random effects model), or a specific population about which inferences are to be made (fixed effects model) (35). Perhaps it is best to examine this problem based on the interpretation of the results rather than the selection of the parents. In this case, Hayman's analysis (13) is based on statistical models with fixed genotypes. Therefore, inferences should pertain to the specific poulation in the diallel and pronouncements about a general population must be made with caution.

In order to validate the statistical measurements, Hayman (13) proposed several assumptions:

1) Diploid segregation;
2) Independent action of non-allelic genes;
3) No multiple allelism;
4) Homozygous parents;
5) Independent distribution of beneficial and deletarious alleles among the parents.

Hayman also included another assumption, i.e., that differences between reciprocal crosses did not exist. But, this assumption was later eliminated because it was not a necessary condition for the successful interpretation of the diallel (19).

Baker (2) has pointed out several potential problems reguarding Hayman's assumptions. He suggested that the independent distribution of alleles among the parents is critical to the proper interpretation of the diallel analysis. However, quantitative traits with many genes rarely adhere to this restriction. Failure may be due to the effects of linkage or from the effects of a limited sampling size. Genes at n loci cannot be independent unless a minimum of 2^n parents are used in the diallel. A polygenic trait would require a sample size far beyond practical application. Hayman (13) also recognized the possible failure of this assumption and suggested that the average degree of dominance may be overestimated. Baker (2) concluded that to assume independent distribution in a polygenic trait is unrealistic.

Another key assumption which may frequently be violated is the presence of epistasis. Hayman (13) tested for epistasis in the diallel by examining the differences between variances and covariances at a specific parental array. However, this test is only valid when the genes are independently distributed among the parents (2). In addition, epistasis may go undetected due to balanced failure of the model (13). In either case, the results in the covariance-variance graph may be skewed because of epistasis or a correlation between the genes.

Hayman's analysis produces estimates of the genetic parameters D, H_1, H_2, and F (13, 18, 21). These estimates indicate the additive variance, the overall degree of dominance, the relative dominance properties of the parents, and the gene distribution in the parental lines. The degree of dominance is demonstrated graphically by the regression of the covariances and variances of parental arrays (W_r on V_r). Definitions of these parameters and their application will be described in the Materials and Methods section.

MATERIALS AND METHODS

Seven wheat lines representing a range of reactions to WSSM were chosen as parents for a diallel mating design. Host reaction to the disease was determined by symptom evaluations in the field and preliminary virus screening work by Haufler and Fulbright (10). The field rating system was based on the presence and severity of WSSM symptoms. A numerical scale from 0 to 5 was used, with a rating of 0 representing resistant lines and a rating of 5 representing susceptible lines. Parents were chosen for virus screening based on their differential field reactions to WSSM. The parents included in the diallel were three cultivars developed in Michigan; Augusta (CI 17831), Ionia (CI 14469), and Tecumseh (CI 17287) and four advanced experimental lines from the wheat breeding program at Michigan State University; B4145, B6018, B7321, and B9028 (Table 1). The seven parents were rated for WSSMV infection using ISEM (12) and were ranked in order of increasing resistance to WSSMV (Table 2), based on the virus concentration found within each parent.

Table 1. Pedigrees of the seven soft winter wheat lines used as parents in the diallel.

Common Name or Experimental No.	Pedigree
Ionia	Redcoat/3*Genesee
Augusta	Genesee/Redcoat//Yorkstar
B4145	Genesee*2/Redcoat//Talbot/3/E4501/Arawa//Hilgendorf
Tecumseh	Minhardi/Wabash/5/Fultz selection/Hungarian/2/W38/3/ Wabash/4/Fairfield/6/Redcoat sib/Wis, CI12633/7/ Vigo/4/Trumbull/2/Hope/Hussar/3/Fulhio/Purkof (Purdue 427a1-1-3)*3/5/Kenya Farmer
B7321	Mironovskaja 808//AC4835/4*Genesee
B6018	CI 9321/2*Genesee//Asosan/3*Genesee/3/Talbot/ CI 9321//Genesee*5/P4217
B9028	Novi Sad-12-56/Bezostaja-1/2/Heine-7/4/Seuwon 92/ Brevor//Yorkwin/3/2*Genesee

Table 2. Winter wheat lines used as parents in a complete diallel to examine WSSMV resistance.

	Experimental or CI no.	Common Name	WSSMV Reaction
1.	CI 14469	Ionia	Susceptible[4]
2.	CI 17831	Augusta	Susceptible
3.	CI 17287	Tecumseh	Moderately susceptible[3]
4.	B4145	Experimental line	Moderately resistant[2]
5.	B7321	Experimental line	Moderately resistant
6.	B6018	Experimental line	Resistant[1]
7.	B9028	Experimental line	Resistant

[1] Resistant lines have neither symptoms nor virus particles.

[2] Moderately resistant lines have very few detectable symptoms and have fewer than 20 particles per grid square (pgs).

[3] Moderately susceptible lines show mild to moderate symptoms. Virus particlecountsare between 20 to 70 pgs.

[4] Susceptible lines have many distinct symptoms and have virus particlecounts of 70 or more pgs.

Infection

Environmental conditions regulate the severity of WSSMV infection in susceptible genotypes. Therefore, in order to accurately evaluate genetic differences, the environment must be controlled to provide an ubiquitous infection potential for all the lines. Temperature fluctuations, vernalization and soil moisture influence infection (16, 26, 27, 37). Since it was not possible to control these variables in the field, a growth chamber was used.

Infested soil was collected from a diseased wheat field at Saranac, MI. Wheat at this location has shown severe WSSM symptoms for several consecutive years. Because the soil was a loamy clay, a mixture of 80% infested soil and 20% sterilized sand was used to improve aeration and soil drainage.

Sterilized wooden flats were filled with the infested soil mixture. Seeds from the seven parental lines and 42 F_1 progenies were randomly planted in rows with 5 seeds per row on November 1, 1983. Each flat had 7 parental rows, 21 F_1 progeny rows, and 2 susceptible checks. (The 42 F_1 progeny seeds were divided into two reciprocal groups of 21 each). Three replications of each of the two reciprocal crosses were planted.

After germination, the seedlings were kept in the greenhouse for 20 days at 20 ± 3 C. The plants were then taken outdoors to a cold frame to vernalize for 90 days. The cold frame was a cinderblock enclosure with a metal hardware cloth covering. After vernalization, seedlings were transferred to a growth chamber at 10 C with 10,000 lux of light for 10 hours and at 8 C during the dark period. Plants were sampled directly from the growth chamber for this study (27, 36).

Sampling

Each replication contained the parents and all of the F_1 progenies, excluding reciprocals. An entire population (one replication) of plants was sampled every three weeks and prepared for viewing with the electron microscope. The replications were blocked by sampling time (weeks). Each entry was sampled at least three times. Lower leaves from several different plants in each row were harvested and weighed. Only the lower leaf of each plant was harvested in order to maintain a uniform sampling procedure. Since the plants produced different amounts of leaf matter in the early sampling periods, a ratio of 0.15 gram leaf tissue (fresh weight) to 1.0 ml ISEM buffer was used for all of the samples.

The leaves were cut into small pieces with scissors and ground with a mortar and pestle in liquid nitrogen. The ground leaf material was then placed into a small vial to which ISEM buffer was added. The buffer suspended the ground tissue into an aqueous suspension. An entire replication was sampled within three days to limit non-genetic variation.

Immunologically Specific Electron Microscopy

Immunologically specific electron microscopy (ISEM) was developed for WSSMV by Haufler and Fulbright in 1983 (10, 11). WSSMV antiserum with a titer of 1/320 in complement fixation was used (36). The Derrick technique (5) as modified by Haufler and Fulbright (10, 11) was used to coat grids with antiserum. Carbon-coated Parlodion-filmed 300-mesh grids were floated on 30 ul drops of a 1:500 dilution of antiserum in 0.06 M Na_2HPO_4-NaH_2PO_4 buffer, pH 7.0. Drops were placed on Parafilm-wrapped microscope slides which were placed in a petri dish containing moistened filter paper. The grids were heat treated at 37 C for three hours, then rinsed twice for ten minutes in ISEM buffer. Rinsed grids were then briefly drained and placed on 30 ul drops of suspended sample extracts at 4 C overnight (approximately 10 hours). During this time, the virus particles in the sample extracts were specifically absorbed onto the WSSMV antiserum-coated grids. For specificity and ease of detection with the electron microscope, the grids were drained and again floated on drops of antiserum (decoration step) for two hours at 4 C, thus sandwiching the virus particles between layers of antiserum (Figure 1). After decoration, grids were drained and negatively stained with 2% ammonium molybdate, pH 7.0. All samples were examined in a Philips 201 transmission electron microscope

SAMPLE PREPARATION

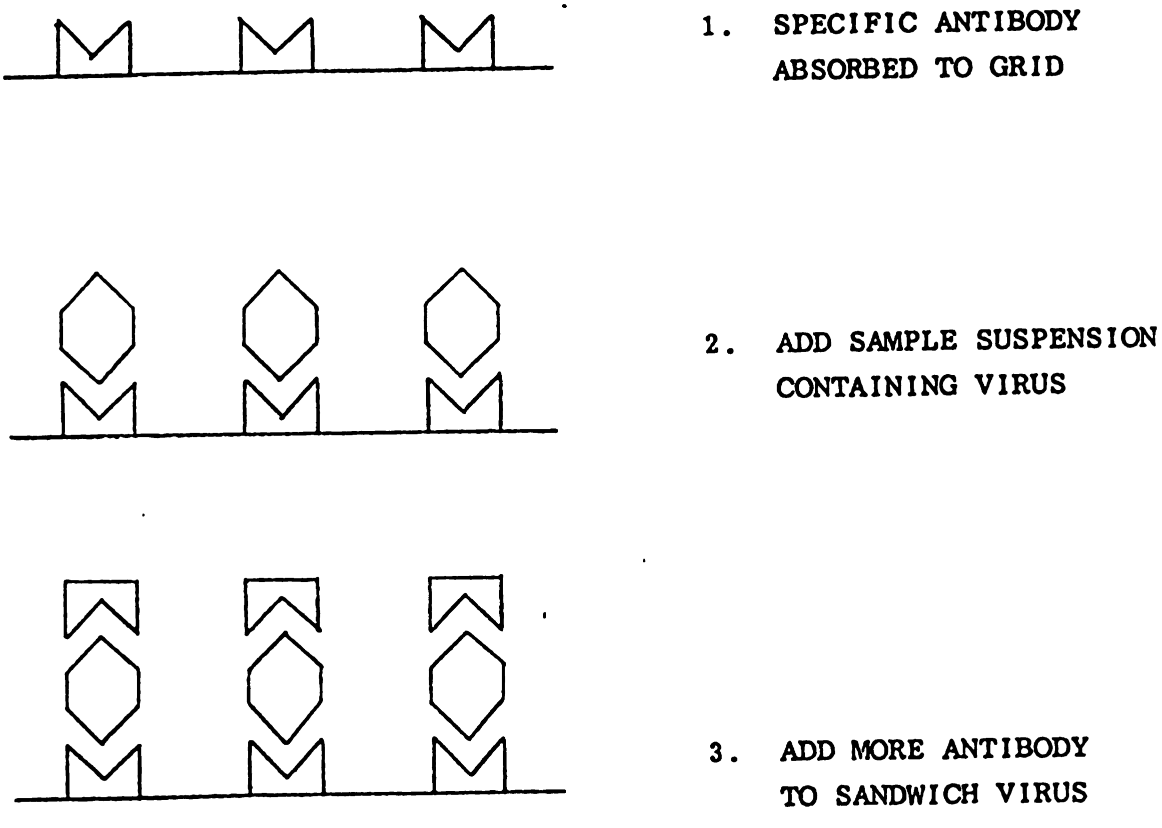

Figure 1. ISSEM methodology for screening virus diseases.

operated at 60 kV. An estimate of the total number of virus particles was made by averaging the particles visable on ten randomly chosen 300-mesh grid squares (particles per grid square, pgs).

Diallel Analysis

As mentioned in the preceding section, the raw data were presented as mean particles per grid square. The data were averaged over samples and analyzed according to Hayman's model (13) using a log transformation (21). Hayman provides the statistics calculated from a diallel table (13); therefore, only the terms will be defined here. The statistics and genetic components of variation are defined as follows:

V_{OLO} : variance of the parents.

V_r : variance of one array (r^{th} array).

V_{ILI} : mean variance of the arrays.

W_r : the covariance between the parents and their offspring in one array (r^{th} array).

W_{OLOI} : the mean covariance between the parents and the arrays.

V_{OLI} : the variance of the means of the arrays.

$M_{LI} - M_{LO}$: the difference between the mean of the parents and the mean of the n^2 progeny.

E : the expected environmental component of variation.

The additional components are genetic:

D : component of variation due to the additive effects of the genes.

H_1 : component of variation due to the dominance effects of the genes.

F_r : the covariation of additive and dominance effects in a single array.

F : the mean of F_r over the arrays.

H_2 : $= H_1 (1-(u-v)^2)$ where
u = proportion of positive genes in the parents
v= proportion of negative genes in the parents
and where $u + v = 1$.

Hayman (13) provides estimates of the genetic parameters from the variances and covariances of the parental arrays. These statistics are calculated directly from the diallel table (Table 3). The variance of a parental array was calculated as follows:

$$V_r = \left\{ \left(\frac{1}{n-1} \right) \left[\sum_i^r \overline{Y}_{ri}^2 - \left(\frac{\sum_i^r Y_{ri}}{n} \right)^2 \right] \right\}$$

where n is the number of parents, r is the rth parental array, and j is the jth experimental unit.

Table 3. The diallel crossing pattern for the seven parents used in the study of inheritance of WSSMV resistance.

	M0300	M0260	B4145	M0280	B6018	B7321	B9028
M0300	M0300 / M0300						
M0260	M0300 / M0260	M0260 / M0260					
B4145	M0300 / B4145	M0260 / B4145	B4145 / B4145				
M0280	M0300 / M0280	M0260 / M0280	B4145 / M0280	M0280 / M0280			
B6018	M0300 / B6018	M0260 / B6018	B4145 / B6018	M0280 / B6018	B6018 / B6018		
B7321	M0300 / B7321	M0260 / B7321	B4145 / B7321	M0280 / B7321	B6018 / B7321	B7321 / B7321	
B9028	M0300 / B9028	M0260 / B9028	B4145 / B9028	M0280 / B9028	B6018 / B9028	B7321 / B9028	B9028 / B9028

Likewise, the covariance for a parental array can be calculated as follows:

$$W_r = \left\{ \left(\frac{1}{n-1} \right) \left[\sum_i^r \bar{Y}_{ri} \bar{Y}_{ii} - \frac{\left(\sum_i^r \bar{Y}_{ri} \right) \left(\sum_i^r \bar{Y}_{ii} \right)}{n} \right] \right\}$$

Similar calculations were made for all of the parental arrays in the diallel table. Variances and covariances for the array means and their differences were also calculated from the parental array totals using the same procedure. After calculating the statistics and genetic parameters, the legitimacy of the additive-dominance model was examined.

The array covariances, variances, and their differences are used to determine the uniformity and validity of the additive-dominance hypothesis postulated (13). This was tested using the following formula:

$$t^2 = \frac{(n-2)(\text{Var. } V_r - \text{Var. } W_r)^2}{4 \text{ Var. } V_r \times \text{Var. } W_r - \text{Cov}^2(V_r, W_r)}$$

with n-2 degrees of freedom.

The limiting parabola of the covariance-variance graph is constructed by plotting the points V_D, W_D; V_r, W_r; and V_{ILI}, W_{OLOI}. The corresponding parental array values of covariances for all observed variances are then placed on the graph.

The regression coefficient, b, is calculated in the usual way, its standard error being approximately (6).

$$S_b^2 = \frac{S^2}{\Sigma X^2} = \frac{\Sigma y^2 - (\Sigma xy)^2 / \Sigma x^2}{(n-2)\,\Sigma x^2}$$

The equation 1/2 Var $(W_r - V_r) = s^2$ was used to estimate the accuracy of the components of variation, and the terms of the main diagonal of the covariance matrix (13) are used as corresponding multipliers. The results of these computations will be discussed in the **Results and Discussion** section.

Fungal Infection

It was important to determine whether resistance to WSSM occurred because the fungal vector was inhibited from infecting the plant or if virus replication was blocked within the host after transmission. The diallel plant populations were used to examine the possibility that resistance to WSSM was due to the inability of *P. graminis* to infect certain lines.

Two seeds for each entry were planted in 4 inch pots containing infested soil. Two replications, each containing the entire diallel population of 7 parents and 21 F_1 progenies were grown in a growth chamber at 15 C. After 4 weeks the plants were removed from their pots, and their roots were gently washed with distilled water, treated by heating in 10% KOH, and acidified in 0.1 M HCl. The samples were then stained in 0.05% trypan blue in lactophenol (24) and examined for the presence of fungal resting spores using a Wild light microscope at 300X magnification. Since the number of resting spores in the roots was difficult to quantify, only the presence or absence of the vector was recorded.

RESULTS AND DISCUSSION

Inheritance of resistance to WSSM was studied by the diallel method of genetic analysis using ISEM to measure the number of virus particles. The raw data is presented in Table 4 as a half diallel since there were no significant maternal effects. This table provides the basis for calculating the variance of the parents, the seven parental arrays, and the covariances of the parental set with their progenies in an individual array. A data transformation was justified by Mather (21), and therefore, the logarithmic scale $y = \log(x + 1)$ was used throughout the analysis to fit the additive-dominance model proposed by Hayman (13).

Before proceeding with the diallel analysis, first consider the preliminary analysis of variance (Table 5). The differences between genotypes are highly significant. This was expected, since the parents were chosen based on their differential reactions to WSSM. The preliminary AOV table indicates extremely high broad-sense heritability for this trait. There is no significant difference between replications which also represents an absence of maternal effects. And, the environmental component of variation, represented by the error mean square, is very small. Since the growth chamber environment offered maximum uniform infections with the least amount of non-genetic variation, all future genetic and statistical inferences pertain to this environment.

Table 4. Virus particle counts (particles per grid square) using ISEM for parents and F_1 progeny in a half diallel, replication 1.

Parental Number and Name		1	2	3	4	5	6	7
1	Augusta	97						
2	Ionia	200	370					
3	B4145	10	4	10				
4	Tecumseh	157	216	4	16			
5	B6018	5	1	0	1	1		
6	B7321	78	86	1	7	0	20	
7	B9028	1	5	1	0	0	0	0

Replication 2.

Parental Number and Name		1	2	3	4	5	6	7
1	Augusta	100						
2	Ionia	166	350					
3	B4145	2	1	11				
4	Tecumseh	133	233	2	15			
5	B6018	2	4	0	1	1		
6	B7321	45	70	1	8	1	18	
7	B9028	0	2	2	0	2	0	0

Table 5. Preliminary analysis of variance of parents and F_1's from virus particle counts using ISEM in the diallel (transformed data).

Source of Variation	df	MS	F	P
Total	55			
Replication	1	.024	.983	> .100
Genotypes	27	1.355	54.612**	< .001
Error	27	.025		

Broad-sense heritability $\dfrac{\text{SS Genotypes}}{\text{SS Total}} = 0.981$

Diallel Analysis

Assured that there are significant genotypic differences for the trait, the statistics provided from the diallel analysis were examined. Table 6 presents the array covariances, variances, and their differences. These calculations are based on the transformed data averaged over replications and reciprocals.

In order to test if the additive-dominance model effectively accounted for the total amount of genetic variation, the covariance - variance data were analysized (Table 7). Neither replications nor arrays are significant in the analysis of variance, so the additive-dominance model with genes independently distributed among the parents is adequate to describe the variation in WSSMV resistance.

Table 6. Table of array covariances, variances, and their diffferences for the parental arrays.

Parents	Covariances (W_r)	Variances (V_r)	$W_r - V_r$
Augusta	0.6198	0.6169	0.0029
Ionia	0.7078	0.8760	- 0.1682
B4145	0.2134	0.1577	0.0557
Tecumseh	0.7741	0.8002	- 0.0261
B6018	0.1255	0.0789	0.0466
B7321	0.7080	0.7054	0.0026
B9028	0.2075	0.0858	0.1217
Totals	3.3561	3.3209	0.0353

Table 7. Analysis of variance for the differences between the covariances and variances of parental arrays ($W_r - V_r$).

Sources of Variation	df.	MS	F	P
Total	13			
Replication	1	0.0188	4.5759	.05 - .10
Arrays	6	0.0166	4.0474	.05 - .10
Error	6	0.0041		

It should be noted that the F values for the analysis of variance lie between the .05 - .10 α-levels of significance, which indicates that some interallelic gene action may be important in the final model.

Confident that this model explains the overall genetic variance, the main statistics were examined:

$E = 0.0248$: the expected environmental component of variation.

$V_{OLO} = 0.7929$: variance of the parents.

$V_{OLI} = 0.2768$: the variance of the means of the arrays.

$V_{ILI} = 0.4780$: mean variance of the arrays.

$W_{OLOI} = 0.4464$: the mean covariance between the parents and the arrays.

$(M_{LI} - M_{LO})^2 = 0.1290$: the difference between the mean of the parents and the mean of the 21 progeny.

By using these main statistics and the corresponding multipliers given by Hayman (13), the components of variation and their standard errors were calculated (Table 8). Both additive variance (D) and dominance variance (H_1) contribute significantly to the overall genetic variance. In addition, $D - H_1$ is not significantly different from zero, indicating complete dominance. The sign of F is an indicator of the relative frequencies of dominant and recessive alleles. For WSSMV resistance, the negative F value indicates a greater frequency of recessive alleles in the parental lines.

Table 8. Components of variation and their standard errors for the diallel.

Notation	Estimate	Standard Error	P
D	0.7681	± .0439	< .001
F	- 0.2351	± .1054	.05-.01
H_1	0.8095	± .1058	< .001
H_2	0.7176	± .0932	< .001
$D - H_1$	- 0.0414	± .0918	> .10
E	0.0248	± .0155	> .10
Proportions			
$\sqrt{H_1/D}$	1.0266		
$H_2/4H_1$	0.2216		
Heritability	0.40		

In addition to these genetic components, the genetic ratios describing gene action, allelic distribution between the parents, and a heritability estimate is also given in Table 8. The mean degree of dominance is $\sqrt{H_1/D} = 1.0266$, denoting nearly complete dominance since a value of 1.0 is equal to complete dominance. This is described in Figure 2 which shows a simple genic system for one locus. One parent is designated by the allelic combination AA and the other parent aa. Typically, the uppercase letters represent dominance while the lowercase letters represent recessive genotypes. We assume that both parents are homozygous for the allele as stipulated by Hayman (13). The letter m represents the mid-parental value, h is the measure of dominance, and d is the measure of additive variance. In the case of complete dominance, the heterozygote equals the value of the dominant parent AA, and d is equal to h. Partial dominance, as shown in Figure 2, has the heterozygote (Aa) falling between the mid-parent and the AA parent. Correspondingly, the h value is less than d. When the values of h and d are summed over all parents and loci for this trait we have the values D and H_1. The square root of H_1/D will equal 1 when the heterozygote is equal to the dominant parent.

This is geometrically represented by the regression line in Figure 3, which does not significantly deviate from the origin of the covariance-variance graph.

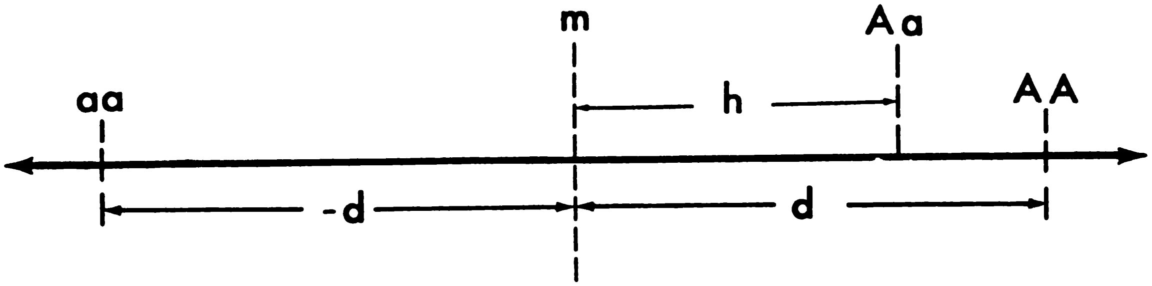

Figure 2, A simple genetic number line describing describing the additive and dominant portions of genetic variance.

The proportion of the genes with positive (u) and negative (v) effects in the parents averaged over all loci is $H_2/4H_1$. The maximum value of 0.25 occurs when the positive and negative alleles appear in equal number (u = v = .5). In this study $H_2/4H_1$ is 0.22, suggesting that there are slightly more loci with negative effects. But, H_2 is not significantly different from H_1 and therefore, the ratio approaches the maximum value of 0.25. Narrow sense heritability is 0.40 (Table 8).

Covariance-Variance Graph

The regression of covariance on variance is geometrically represented by Figure 3. The graph reveals that the pattern of inheritance of resistance to WSSMV is one of complete dominance uncomplicated by the failure of any of the earlier assumptions. Plotting the parental W_r/V_r points on the graph reveals an order of dominance among the parental arrays. Those parents nearest the origin (B6018, B9028, and B4145) contain a greater proportion of dominant alleles for resistance to WSSMV. The parents further up the regression line contain a greater proportion of recessive alleles and are more susceptible to WSSMV (B7321, Tecumseh, Augusta, and Ionia). The parents are grouped into two distinct patterns. The importance of these patterns will be examined in the proposed genic model later in this section.

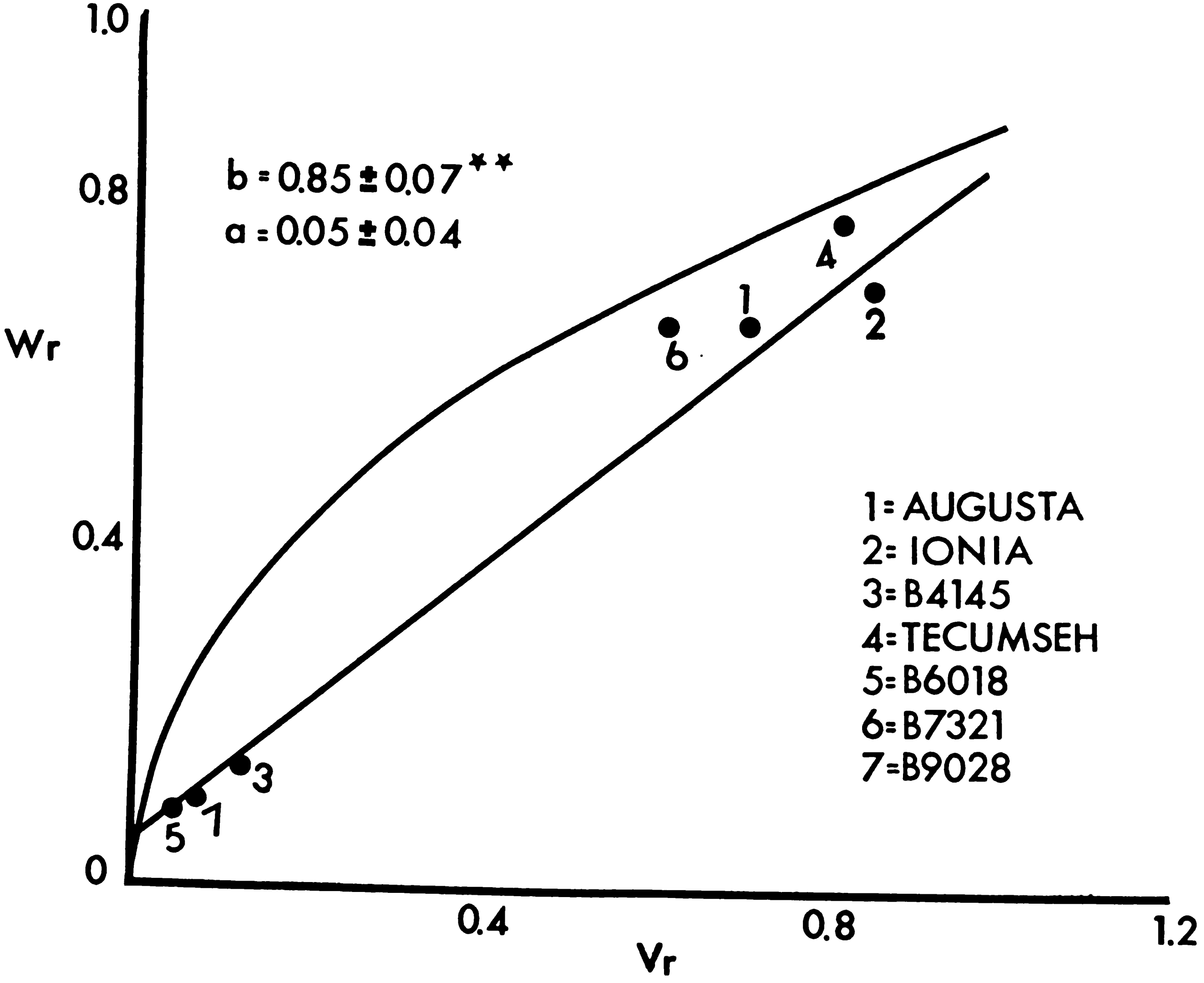

Figure 3. The covariance-variance graph for the parental arrays of the seven parent diallel.

The correlation between calculated (covariance plus variance) and observed parental order of dominance is $r = 0.81$, and the coefficient of determination is $r^2 = 0.61$. This significant positive correlation indicates that genes for resistance to WSSM are mostly dominant. The test for the deviation of the regression line from unity, $t^2 = 2.20$, is not significant with 5 degrees of freedom which gives further support to the original hypotheses that describes the overall genetic variation in terms of additive and dominant variance.

The regression of calculated and observed parental order of dominance can also be used to predict the measurements of completely dominant and recessive parents. These predictions suggest the theoretical limits of selection among genes showing dominance and were found to be - 0.754 and 2.78. This is the range between upper and lower limits and can be used to estimate the number of loci. The results are very tentative, since they are based on three conditions which are often not met: (1) all the favorable alleles are fixed at both limits; (2) all the genes have equal effects; and (3) all the genes have initial frequencies of 0.5.

The formula used to compute the estimate of the number of loci is:

$$\frac{R^2}{\sigma_A^2} = 8n \quad \text{or} \quad n = 2.04$$

where R^2 is equal to the square of the difference between upper and lower limits, σ_A^2 is the additive variance of the initial population, and n is the number of loci (7). Since the assumptions may not all be valid, and the correlation coefficient is not near unity, this estimate is prone to error.

Allelic Model

The parents used in the diallel fell into six distinct parental classes based on virus particle counts. A two-locus genetic model is hereby proposed to account for the observed classification of parents and the patterns of inheritance exhibited in the F_1 progenies. The model (Table 9) has three alleles (A_1, A_2, and A_3) at the A locus and two (B_1 and B_2) at the B locus. The A_1 allele is completely dominant to A_2 and A_3 and codes for resistance to WSSMV. The A_2 and A_3 alleles show additive gene action, so that parents with the A_3 allele are more susceptible than parents containing the A_2 allele. The B locus also exhibits additive gene action, so that parents with homozygous B_2 alleles are more susceptible to WSSMV than parents containing homozygous B_1 alleles. The heterozygote B_1B_2 lies nearly midway between the homozygous parents expressing additivity. In addition, there is an additive by additive epistatic interaction between loci A and B in the absence of the A_1 allele.

GENIC MODEL

	$A_1A_1B_1B_1$	$A_1A_1B_2B_2$	$A_2A_2B_1B_1$	$A_3A_3B_1B_1$	$A_2A_2B_2B_2$	$A_3A_3B_2B_2$
$A_1A_1B_1B_1$	1					
$A_1A_1B_2B_2$	1	11				
$A_2A_2B_1B_1$	1	1	19			
$A_3A_3B_1B_1$	1	3	8	16		
$A_2A_2B_2B_2$	2	6	61	145	99	
$A_3A_3B_2B_2$	6	3	78	225	183	360
PARENTS	B6018 B9028	B4145	B7321	TECUMSEH	AUGUSTA	IONIA

Table 9. Predictive model proposing allelic combinations to represent the phenotypic variation. (values given as an average number of virus particles per grid square).

The presentation of this model differs from the Hayman analysis at two important points. Namely, the presence of multiple alleles at locus A, and the epistatic gene action between loci A an B in the absence of allele A_1. Hayman (13) briefly discusses these points and offers some possible explanations to compensate for their presence. Multiple allelism can be a very complicated matter. However, the F_1 analysis should not be greatly influenced by the presence of multiple alleles, because in the absence of segregation it is the same as polygenic biallelism. The F_2 analysis will present complications due to segregation, but these differences can be predicted by the model and act as a test of the hypothesis' validity.

Epistatic gene action also can be a very complicated matter. The homogeneity of the covariance-variance analysis suggests that there is no epistatic gene action. However, homogeneity may be attained in certain cases by balanced failure (13). This type II error is impossible to detect, but it is interesting to note that the diallel analysis of the raw data indicated that one or more of the underlying assumptions was incorrect and suggests some interallelic gene action. Recall also that in the analysis of differences between array covariances and variances for the transformed data, both replications and genotypes were significant sources of variation at the .10 α-levels (Table 7). Perhaps type II error occurs in the analysis of the transformed data so that significant differences are not detected.

The proposed model can be tested by analyzing disease reactions among plants in the segregating generations. Such an analysis will offer more insight into the true genetic system. The model could be tested by examining key F_1 progenies for their segregation in the F_2. Progenies with genotypes that are heterozygous at only one allele should be tested against the model's predictions for the F_2 segregation ratios. For example, the genotype $A_1A_2B_1B_1$ should yield a 3:1 ratio of resistant offspring to moderately resistant offspring. A more complex study would also examine progenies that were heterozygous at both alleles. The genotype $A_2A_3B_1B_2$ would give nine different classes, although some of the phenotypes are not statistically distinguishable. The results of the observed segregation ratios can be compared to the model's predicted ratios in order to evaluate the model. The two-gene model is a testable hypothesis.

Resistance to the Fungus

The second experiment examined whether or not resistance to WSSMV might be due to resistance to the fungal vector. All the genotypes from the diallel were examined for the presence of the fungal vector, *Polymyxa graminis*. All lines, including the resistant material, tested positive for the presence of the fungus. This suggested that resistance is not to the fungus vector but must either inhibit transmission of the virus or replication within the host. It is possible that different genetic systems control both of these biological functions, but this study did not provide enough evidence to make a hypothesis concerning the means of resistance.

SUMMARY AND CONCLUSIONS

Inheritance of resistance to WSSMV was studied by examining parental and F_1 populations from a seven parent diallel. The plants were tested for the presence of virus particles using ISEM as described by Haufler and Fulbright (10).

1. Plant reaction to WSSMV infection showed contiuous phenotypic variation.

2. No reciprocal differences were found in the diallel analysis.

3. The simple additive-dominance model proposed by Hayman (11) adequately described the genetic variation in resistance to WSSMV when a log transformation was used.

4. Analysis of the diallel showed that resistance is controlled by completely dominant genes with some additive effect.

5. A model based on the distribution of F_1 progenies and parents suggests a more complex genetic system. There are two genes, locus A which has three alleles and locus B which has two alleles. Allele A_1 is completely dominant for resistance to WSSMV. In the absence of the A_1 allele, alleles A_2, A_3, B_1, and B_2 have additive gene action; and there is an additive by additive epistatic interaction between loci A and B.

6. WSSMV resistance is a qualitative trait controlled by only a few genes. The continuous variation present in the field is a result of the interaction of three complex genetic systems: the vector, the virus and the host.

7. WSSMV resistance is highly heritable and has high penetrance.

8. WSSMV resistance is not due to resistance to the fungal vector *Polymyxa graminis*.

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